stability of the employed oligonucleotide nanolevers under different electrolyte conditions. Temperature dependent data are presented from 4° to 70°C for a range of different bacterial and human proteins: while some show a straightforward unfolding transition, others feature more complex signatures which are indicative of the protein expansion before an unfolding of individual domains sets in at higher temperatures. We discuss the possibility to evaluate quantitative thermodynamic parameters (melting points, transition energies) from the molecular dynamics data and compare our results to established methods like thermofluor assays.
Since the presented approach works on a chip surface it uses minimal amounts of sample and can be performed in parallel using a microelectrode array. In addition, it yields binding kinetics data (association and dissociation rates) and affinity values and thus is ideally suited for investigating interactions in combination with a thermodynamic characterization of the involved proteins.

112-Plat
Evaluating Intracellular Crowded with a Glycine-Inserted Mutant Fluorescent Protein
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The cell environment is very crowded, containing various molecules, proteins and nucleotides. This crowded condition is an indispensable factor for cellular functions of proteins. In the past, the diffusion coefficient of a chemical probe has been used as an evaluation index of the intracellular crowded condition. However, crowding depends not only on the mobility, but also the density of the crowding agents. We have succeeded in making a yellow fluorescent protein (YFP) that senses crowding density via hydrophobicity by inserting into the YFP a glycine and conjugating it to cyan fluorescent protein (CFP), which is insensitive to Förster resonance energy transfer (FRET) probe. This probe has been named GimRET (Glycine inserted mutant FRET probe). GimRET enabled us to visualize the dynamic changes of the intracellular crowding density during cell division. Because GimRET can distinguish the crowding density from the viscosity of the solution, crowding can be evaluated by the GimRET fluorescence intensity ratio and diffusion coefficient, which respectively reflects the density and mobility of the crowding agents. While the diffusion coefficient of GimRET linearly relates to the fluorescence intensity ratio, the slope also depends on the location of the cell, i.e., nucleus or cytoplasm, indicating that the diffusion coefficient alone is insufficient for defining crowding. Here, we propose the simultaneous observation of the GimRET intensity ratio and the diffusion coefficient as a way to evaluate intracellular crowding.

113-Plat
NIR Fluorescent Proteins with Synthetic Chromophores for Deep Tissue Imaging
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Nearly all existing genetically encoded fluorescent tags prove inadequate for imaging deep within tissues and small animals due to the overwhelming absorbance, scatter, and autofluorescence contributed by native chromophores. While the majority of such interference is confined to the visible spectrum of light, few genetically encoded probes operate in the near infrared (NIR) where tissues are most optically transparent. We aim to generate a toolbox of NIR-emitting fluorescent proteins that utilize synthetic chromophores capable of reporting physiological parameters. These chromophores, which we call fluorogens, are non-fluorescent until incorporated into their cognate protein (an scFv derivative). We have shown that existing fluorogens can be systemically delivered in animals via intraperitoneal or intravenous injection to generate a fluorescent signal exclusively at the site of cognate protein expression. To date, we have discovered and characterized two protein:fluorogen pairs that produce easily separable colors appropriate for deep tissue imaging, with excitation and emission maxima at 702/731 nm and 744/764 nm, respectively.
This system promises a degree of modularity unrivalled by traditional fluorescent proteins, as specific sites on each fluorogen can be manipulated without greatly perturbing its ability to bind to its cognate protein. Utilizing this feature, we have developed cell permeant and impermeant fluorogens, the latter allowing investigators to selectively interrogate targets such as membrane proteins that are exposed to the extracellular environment. Further efforts will focus upon the development of new fluorogens that expand the available palette of separable NIR colors, minimize cross-labeling between structurally distinct fluorogens, and incorporate the ability to detect and report upon environmental conditions such as pH and other ion concentrations.

114-Plat
Genetically Encoded Gas Nanostructures as Biophyically Tunable Molecular Reporters for MRI and Ultrasound
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The study of cellular and molecular processes occurring deep inside living organisms requires new technologies for non-invasive molecular imaging. In particular, there is a need for ‘‘magnetic’’ and ‘‘acoustic’’ analogs of the green fluorescent protein (GFP) that can be used to sensitively observe gene expression using magnetic resonance imaging (MRI) and ultrasound. We are developing genetic reporters for both of these modalities based on the unique biophysical properties of genetically encoded gas nanostructures known as gas vesicles (GVs). Expressed by aquatic microorganism as a means to control buoyancy, GVs are hollow protein-shelled nano-compartments that exclude water but are permeable to gas. We have adapted GVs as the first genetically encoded reporters for hyperpolarized MRI - a form of imaging in which nuclei such as the biocompatible noble gas xenon are introduced into tissues in a non-equilibrium state with 104 - 105 stronger polarization compared to conventional 1H-MRI. Xenon partitioning into GVs enables their detection using chemical exchange saturation transfer at sub-nanomolar reporter concentrations. In parallel, we have shown that GVs can be detected with high frequency ultrasound, their physical properties enabling linear, harmonic and collapse-mode imaging in vitro and in vivo. Furthermore, inter-species differences in the genetically encoded biophysical properties of gas vesicles enable multiplexed imaging with both MRI and ultrasound and provide clues for genetic-level biophysical tuning of these unique nanostructures.

115-Plat
Label-Free Optical Detection and Super-Resolution Microscopy of Single Proteins
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Sensitive detection of biological entities is of central importance for laboratory and clinical research as well as public health, environmental monitoring and pharmaceutical industry. In the past decade, many groups have pursued sophisticated methods to achieve label-free detection and sensing of single macromolecules, for example by using plasmonic nanostructures or optical microcavities. We show that simple extinction measurements have the intrinsic sensitivity to directly detect small proteins. The underlying mechanism of our technique is the interference between a laser beam and the light that is coherently scattered by the analyte. By combining this method with microfluidics and surface functionalization of a substrate, we demonstrate that it is possible to detect and image a single small protein in real time and distinguish it from the large background of other scatterers [1]. In addition to its application in ultrasensitive label-free biosensing, our work paves the way for fluorescence-free super-resolution imaging of the interaction between individual proteins.
[1] M. Piliarik, V. Sandoghdar, under review.

Platform: Membrane Receptors and Signal Transduction I

116-Plat
Direct, Single Molecule, Cell-by-Cell Observation of Molecular Kinetics and Thermodynamics in Early Lymphocyte Signaling
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T cells exhibit near-single-molecule sensitivity for agonist peptide fragments in a background of thousands of structurally-similar self peptides. Unraveling the physical mechanism of this extreme molecular sensitivity and specificity would inform our understanding of the immune system’s role in disease and potentially reveal universal cell signaling processes. We study the T cell signaling pathway with a highly controlled supported lipid bilayer-live cell junction. Using this system and a multi-timescale imaging approach we report direct, single molecule observations of T cell receptor-peptide-MHC binding kinetics in living T cells. Direct, cell-by-cell measurements of binding kinetics yield unexpected values for dissociation constants and kinase recruitment to the membrane as a function of peptide density and quality.